Interleukin 7 (IL-7) selectively promotes mouse and human IL-17-producing cells

Interleukin 7 (IL-7) selectively promotes mouse and human IL-17–producing γδ cells

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IL-17–producing CD27$^-$ γδ cells (γδ^{27-} cells) are widely viewed as innate immune cells that make critical contributions to host protection and autoimmunity. However, factors that promote them over IFN-γ–producing γδ^{27+} cells are poorly elucidated. Moreover, although human IL-17–producing γδ cells are commonly implicated in inflammation, such cells themselves have proved difficult to isolate and characterize. Here, murine γδ^{27-} T cells and thymocytes are shown to be rapidly and substantially expanded by IL-7 in vitro and in vivo. This selectivity owes in substantial part to the capacity of IL-7 to activate STAT3 in such cells. Additionally, IL-7 promotes strong responses of IL-17–producing γδ cells to TCR agonists, thus reemphasizing the cells' adaptive and innate potentials. Moreover, human IL-17–producing γδ cells are also substantially expanded by IL-7 plus TCR agonists. Hence, IL-7 has a conserved potential to preferentially regulate IL-17–producing γδ cells, with both biological and clinical implications.

lymphocytes | cytokines | proliferation

Studies of IL-17 intensified with the identification of a specific subset of CD4$^+$ Th17 cells that upon activation primarily produces IL-17 as opposed to IFN-γ (Th1 cells), IL-4 (Th2 cells), or IL-10/TGFβ (Treg cells) (1–3). Th17 differentiation is regulated by transcription factors RORγt and STAT3, the latter in part explaining the promotion of Th17 differentiation by IL-6 and IL-23 (3). Paradoxically, more detailed studies of Th17 immunity have identified γδ T cells and/or innate-like lymphoid cells as critical initial producers of IL-17 (4, 5). At steady-state, γδ cells are only a minor subset of T lymphocytes, but upon infection by *Listeria*, *Mycobacteria*, or *Plasmodium*, or upon LPS administration, they expand and make critical contributions to host protection (5–9). They likewise underpin immunopathology in widely used models of inflammatory disease (10, 11). In humans, IL-17 protects against mucocutaneous candidiasis and is again implicated in autoimmune inflammation, including psoriasis, multiple sclerosis, and rheumatoid arthritis (12, 13). Hence, there is considerable interest in identifying factors that regulate IL-17–producing γδ cells in mice and humans.

Adding to this interest is the emergence of murine γδ cells as prime examples of thymic preprogramming, whereby functional distinctions between CD27$^+$IFN-γ producers (γδ^{27+} cells) and CD27$^-$IL-17–producing (γδ^{27-}) cells are established by developmental cues that are largely uneludicated (8, 14). For example, γδ^{27-} cells seem largely to arise from fetal thymocytes, requiring neither engagement of cognate ligand, nor RORγt or STAT3 that are both required for TCRαβ$^+$ Th17 cell development (15). However, despite the dispensability of RORγt and STAT3 in development, most peripheral IL-17–producing γδ cells express RORγt and respond rapidly to IL-23 that signals via STAT3 (10). Such rapid responsiveness in the absence of TCR stimulation has led many to classify γδ^{27-} cells as innate immune cells: Indeed, they generally respond poorly to concentrations of TCR agonists that would promote robust activation of γδ^{27+} cells (9). Nonetheless, assigning IL-17–producing γδ cells to innate immunity seems premature until more is known about what regulates the cells and how that might influence their response to TCR stimulation.

Although IL-17–producing γδ cells are likewise commonly evoked in human immune responses and immunopathologies, very little is known about these cells, because they have proved particularly hard to isolate and characterize (16). Thus, it seemed logical that by elucidating stimuli for murine γδ^{27-} cells, one might identify the means to expand their human counterparts. This study identifies IL-7 as a profound and selective activator of IL-17–producing γδ cells in mouse and in human neonates.

Results

IL-7 Enriches for Lymph Node γδ^{27-} Cells. Lymph node (LN) γδ^{27+} cells appear like naïve conventional T cells, being primarily CD62L$^+$ CD25$^-$ CD44^{lo} ICOS$^-$, whereas between 50% and 75% of γδ^{27-} cells resemble activated T cells (CD62L$^-$ CD25$^{+/-}$ CD44^{hi} ICOS$^+$), although they are largely CD69$^-$ (Fig. 1*A* and Fig. S1*A*). As was reported (17), γδ^{27-} cells also express higher levels of IL-7R than do γδ^{27+} cells (Fig. 1*A*). To determine whether this phenotype had functional implications, LN cells were cultured with IL-7 for 4 d. Over eight independent experiments, γδ^{27-} cells were strikingly enriched ∼five- to sevenfold relative to γδ^{27+} cells and ∼6- to 10-fold relative to total LN cells, whereas αβT-cell numbers declined (Fig. 1*B* and Fig. S1 *B* and *C*). Essentially all γδ^{27-} cells were TCR^{hi} CD44^{hi}, and now ∼70% expressed CD69 (Fig. 1 *B* and *C*). IL-7 also increased the proportion of γδ^{27+} cells expressing CD44 and CD69 (Fig. 1*C*), although their numbers declined ∼70% over 4 d, whereas absolute numbers of γδ^{27-} cells increased three- to fourfold (Fig. 1*D*). Strikingly, this enrichment was for cells with IL-17–producing capacity, whose representation increased from ∼30% to ∼70% of the γδ^{27-} subset (Fig. 1*E*). Consistent with this increase, IL-7 enriched for cells expressing RORγt protein but not for those expressing T-bet, a primary regulator of IFN-γ (Fig. S1*D*).

To probe the generality of these observations, we investigated cells from the peritoneal cavity, known to harbor IL-17–producing γδ T cells (18, 19). Ex vivo almost all γδ cells were CD44^{hi} (Fig. S1*E*), and they were enriched after 4 d in IL-7, compared with total cells (Fig. S1 *E* and *F*). However, whereas IL-7 maintained γδ^{27+} cell numbers in vitro relative to culture in medium alone, absolute numbers of γδ^{27-} cells were again increased: ∼ninefold relative to medium alone, and ∼fourfold relative to numbers harvested ex vivo (Fig. S1*G*). Among these cells, the proportion of IL-17 producers was again increased (Fig. S1*H*). Thus, IL-7 preferentially enriches for IL-17–competent γδ T cells from two distinct anatomical sources.

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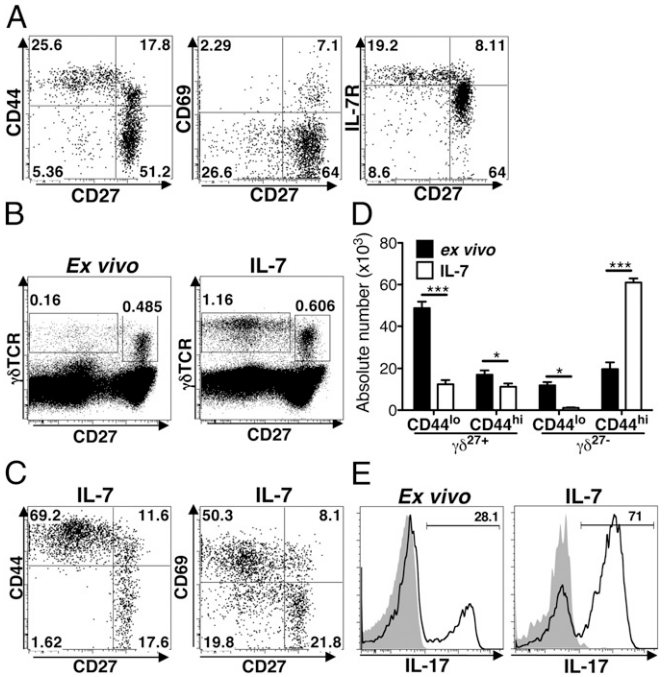

Fig. 1. IL-7 enriches for IL-17–competent γδ T cells. (*A*) γδ T cells from lymph nodes (LN) of adult mice, stained for CD44, CD69, IL-7R, and CD27. (*B*) Representative plots (from $n = 8$ experiments) of total LN cells, ex vivo (*Left*) and after 4-d culture in vitro with IL-7 (*Right*). (*C*) Surface staining of γδ T cells after 4-d culture in vitro with IL-7. (*D*) Absolute numbers of $CD44^{lo}$ and $CD44^{hi}$ $\gamma\delta^{27+}$ cells and $CD44^{lo}$ and $CD44^{hi}$ $\gamma\delta^{27-}$ cells from LN cells cultured as in *B*. Error bars are SEM from $n = 8$ experiments: $^{*}P < 0.05$, $^{***}P < 0.0005$. (*E*) Intracellular staining for IL-17 in γδ T cells from LN cells cultured as in *B* and then activated with PMA + ionomycin.

To examine whether the same effects would be achieved in vivo, mice were administered recombinant IL-7 three times over 5 d and then examined on day 7. There was a conspicuous enrichment of $CD44^{hi}$ $\gamma\delta^{27-}$ cells, with absolute numbers of LN γδ cells competent to make IL-17 upon activation increasing >fivefold, compared with two- to threefold increases in IFN-γ–competent cells (Fig. 2 *A*–*C*). Note that before and after IL-7 treatment, few γδ cells coproduced IL-17 and IFN-γ, consistent with developmental preprogramming (8).

To test whether IL-7 is required for the expansion of IL17–producing γδ cells in vivo, we examined mice treated epicutaneously with imiquimod (IMQ) in which the development of acute psoriaform lesions is largely attributable to expansion of IL-17–producing γδ cells in the skin and skin-draining LNs (11, 20). Indeed, such lesions are comparable in WT and αβ T-cell–deficient mice but dramatically reduced in $TCR\delta^{-/-}$ mice (11, 20). Administration of anti–IL-7R antibody almost completely blocked the enrichment (~10-fold) in IL-17^{+} γδ cells in the skin-draining LNs of mice administered IMQ versus vaseline but did not significantly limit the two- to threefold expansion of IFN-γ^{+} γδ cells (Fig. 2 *D* and *E*). Skin erythema scores, which compose a highly reproducible marker of IMQ-induced pathology, were significantly reduced in anti–IL-7R–treated animals (Fig. 2*F*), as was epidermal thickening that is associated with dermal IL-17–producing γδ cell expansion (11, 20). That some reddening nonetheless occurred most likely reflects widely acknowledged nonimmunological effects of IMQ (20).

Mechanism of Enrichment. Directly ex vivo, few $\gamma\delta^{27+}$ and $\gamma\delta^{27-}$ cells were dividing as judged by Ki67 staining, but after 4 d in IL-7, >90% of $\gamma\delta^{27-}$ cells were dividing compared with only ~30% for $\gamma\delta^{27+}$ cells (black versus gray lines; Fig. 3*A*). Furthermore, when cells were labeled ex vivo with a membrane-intercalating dye, carboxy-fluorescein diacetate succinimidyl ester (CFSE), $\gamma\delta^{27-}$ cells showed much greater dye dilution (by cell division) than did $\gamma\delta^{27+}$ cells (Fig. 3*B*), and it was those dividing cells that accounted for almost all IL-17 production upon stimulation (Fig. 3*C*). Hence, IL-7 drives the preferential expansion of $\gamma\delta^{27-}$ cells with, by contrast, little evidence of selective survival: indeed, *Bcl-2* mRNA whose up-regulation has been associated with antiapoptotic effects of IL-7 in T cells (21) was more strongly expressed by $\gamma\delta^{27+}$ cells (Fig. S2*A*).

IL-7 signals are primarily transduced by STAT5 and PI3-kinase (22–24). However, IL-7–dependent STAT5 phosphorylation was comparable among $\gamma\delta^{27+}$ cells and $\gamma\delta^{27-}$ cells (Fig. 3*D*),

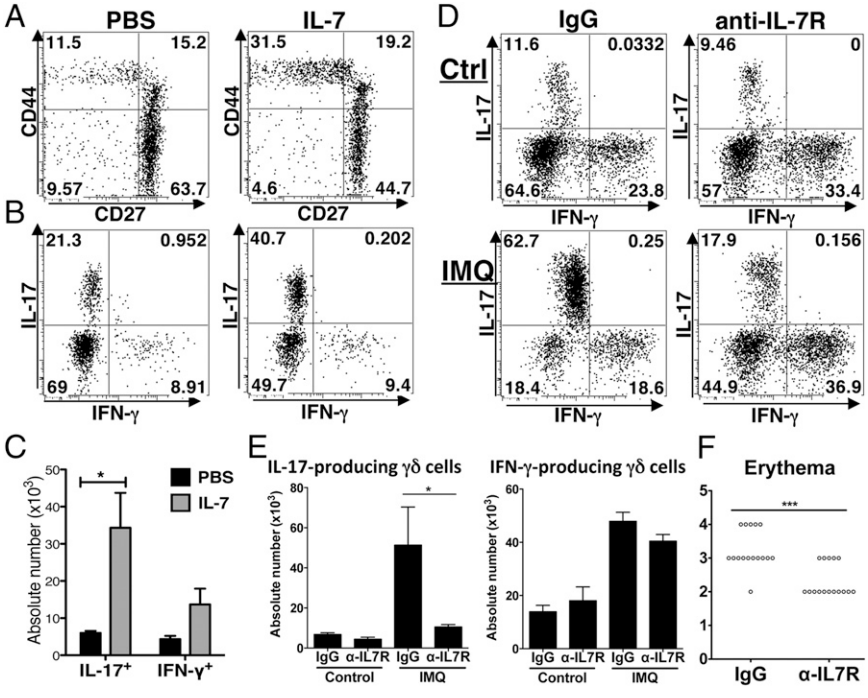

Fig. 2. IL-7 enriches in vivo for IL-17–competent γδ T cells. (*A*) Representative plots ($n = 3$) of γδ T cells from total adult LNs from mice treated with PBS (*Left*) and IL-7 (*Right*). For all plots, numbers indicate percent of cells in relevant quadrant. (*B*) Intracellular staining for IL-17 and IFN-γ in gated γδ T cells, from mice treated as in *A*, and then activated in vitro with PMA + ionomycin. (*C*) Absolute numbers of IL-17^{+} and IFN-γ^{+} γδ T cells from mice treated as in *B*: Error bars are SEM from $n = 3$ mice; $^{*}P < 0.05$. (*D*) Mice were treated with Vaseline (Ctrl; *Upper*) or imiquimod (IMQ; *Lower*) and anti–IL-7R Ab (*Right*) or isotype control (*Left*). Intracellular staining for IL-17 and IFN-γ in γδ T cells from LN cells, activated with PMA + ionomycin. (*E*) Absolute numbers of IL-17^{+} and IFN-γ^{+} γδ cells obtained as described in *D* (error bars are SEM from two experiments, $n = 8$ mice in total, $^{*}P < 0.05$). (*F*) Score for skin erythema from mice treated with IMQ as in *D* (from 4 experiments, $n = 16$ mice in total, $P < 0.005$).

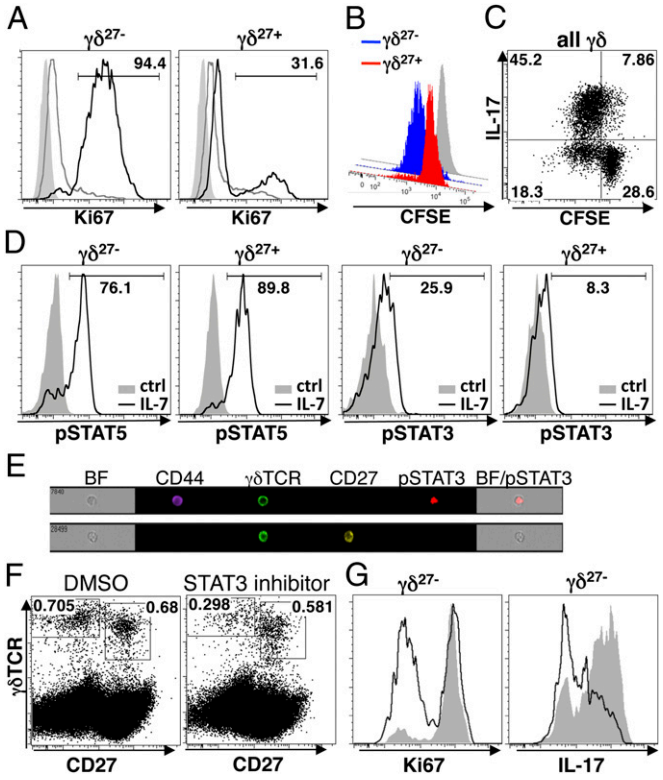

Fig. 3. IL-7 promotes expansion of IL-17–competent γδ T cells via selective STAT3 activation. (*A*) Staining for Ki67 (cells in cycle) in gated $\gamma\delta^{27-}$ (*Left*) and $\gamma\delta^{27+}$ (*Right*) LN cells ex vivo (gray line) and after 4-d culture with IL-7 (black line). Shaded histograms show Ki67 isotype staining. (*B*) Offset histograms of $\gamma\delta^{27+}$ (red) and $\gamma\delta^{27-}$ (blue) LN cells labeled with CFSE and then cultured for 4 d with IL-7. Shaded gray area represents γδ T cells stained ex vivo. (*C*) CFSE-labeled LN cells were cultured for 4 d with IL-7, activated with PMA + ionomycin and stained for intracellular IL-17 and gated on γδ cells. (*D*) Flow cytometric detection of intracellular pSTAT5 and pSTAT3 in gated $\gamma\delta^{27-}$ and $\gamma\delta^{27+}$ cells as labeled. Open and shaded areas indicate IL-7 treatment and controls, respectively. (*E*) Intracellular localization by ImageStream Flow Cytometry of pSTAT3 among two representative $CD44^{hi}$ $\gamma\delta^{27-}$ (*Upper*) and $CD44^{lo}$ $\gamma\delta^{27+}$ (*Lower*) LN cells after 30-min culture with IL-7 (BF, Bright field). (*F*) LN cells preincubated with a specific STAT3 inhibitor and subsequently cultured with IL-7 for 72 h. Representative plots (from $n = 3$ experiments). (*G*) Staining for Ki67 and intracellular IL-17 in gated γδ T cells cultured as in *F*; open and shaded areas indicate STAT3 inhibitor preincubation and controls, respectively. For all plots, numbers indicate percent of cells in relevant gate or quadrant.

failing to account for the differential effects of IL-7 on $\gamma\delta^{27-}$ cells. In fact, STAT5 activation antagonizes Th17 differentiation (25) in which regard the promotion of IL-17–producing cells by IL-7 seemed paradoxical. However, IL-7 may also activate STAT3 (22) which mediates the effects of cytokines known to promote IL-17–producing γδ T cells (10): indeed, after IL-7 stimulation $\gamma\delta^{27-}$ cells showed >threefold higher phospho-STAT3 expression than $\gamma\delta^{27+}$ cells (Fig. 3*D*). This phosphorylation was largely limited to $CD44^{hi}$ cells, in which most pSTAT3 was nuclear, as illustrated by colocalization with propidium iodide (Fig. 3*E* and Fig. S2*C*). In Il17aCreR26ReYFP "fate mapping" mice, cells transcribing the *Il17a* locus induce *cre* that excises a stop codon, thereby irreversibly activating an enhanced yellow fluorescent protein (eYFP) gene in the *rosa26* locus (26). LN $eYFP^+$ γδ cells are $CD44^{hi}$ and $ROR\gamma t^+$ (Fig. S2*D*), and after 30-min stimulation with IL-7, pSTAT3 was selectively expressed by $eYFP^+$ γδ cells (Fig. S2*E*).

When LN cells were incubated for 3 d with IL-7 in the presence or absence of an inhibitor that blocks STAT3 phosphorylation but leaves STAT5 phosphorylation intact (Fig. S2*F*), $\gamma\delta^{27+}$ cells were little affected, whereas the preferential enrichment of $\gamma\delta^{27-}$ cells was reduced by >50%, with a corresponding reduction in $Ki67^+$ cells, and very severe attenuation of cells with IL-17–producing potential (Fig. 3 *F* and *G* and Fig. S2*G*). This effect was not attributable to any toxicity of the inhibitor; for example, $\gamma\delta^{27-}$ annexin-V profiles were equivalent with or without it (Fig. S2*H*). Correlating with the selective IL-7–mediated activation of STAT3 in $\gamma\delta^{27-}$ cells were very low levels of the STAT3 suppressor, SOCS3, relative to $CD44^{lo}$ $\gamma\delta^{27+}$ cells (Fig. S2*I*). Interestingly, the minor $CD44^{hi}\gamma\delta^{27+}$ subset also expressed low levels of SOCS3, perhaps accounting for the maintenance of these cells in IL-7 compared with the loss of bulk $\gamma\delta^{27+}$ cells (Fig. 1*D*).

IL-7 Enriches for $\gamma\delta^{27-}$ Thymocytes. IL-17–producing $\gamma\delta^{27-}$ cells reportedly arise during fetal thymic development (4, 18), although

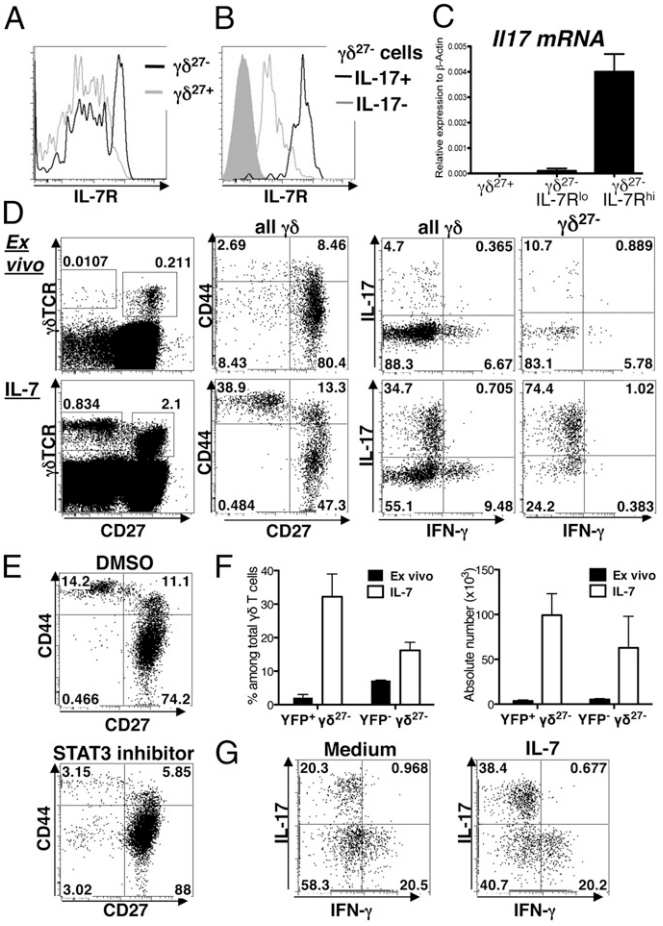

Fig. 4. IL-7 enriches for IL-17–competent γδ thymocytes. (*A* and *B*) Histograms for IL-7R staining of: adult $\gamma\delta^{27-}$ (black line) and $\gamma\delta^{27+}$ (gray line) thymocytes ex vivo (*A*); $\gamma\delta^{27-}$ cells expressing IL-17 (black line) or not expressing $IL\text{-}17^+$ (gray line) after PMA + ionomycin activation (*B*). Gray shaded area is isotype control staining. (*C*) *Il17* mRNA levels in sorted $\gamma\delta^{27+}$, and $IL\text{-}7R^{lo}$ and $IL\text{-}7R^{hi}$ $\gamma\delta^{27-}$ thymocytes determined by real-time RT-PCR. (*D*) Total thymocytes from adult mice ex vivo or activated for 4 d in vitro with IL-7 (*Left*); CD44 and CD27 expression among gated γδ T cells (*Center Left*); Intracellular staining for IFN-γ and IL-17 in all γδ T cells (*Center Right*) and $\gamma\delta^{27-}$ cells (*Right*) after PMA + ionomycin activation. For all plots, numbers indicate percent of cells in relevant gate or quadrant. (*E*) Adult thymocytes preincubated with a specific STAT3 inhibitor (*Lower*) or vehicle control (*Upper*) and subsequently cultured with IL-7 for 72 h. Representative plots (from $n = 3$ experiments) of gated γδ T cells. (*F*) Percentage and absolute numbers of $eYFP^+$ $\gamma\delta^{27-}$ and $eYFP^-$ $\gamma\delta^{27-}$ thymocytes from adult *Il17a*-CreR26ReYFP mice ex vivo (black bars) or after culture with IL-7 for 4 d (open bars). (*G*) γδ cells stained for IL-17 and IFN-γ after 7-d FTOC from embryonic day 16.5 fetal thymus in the presence (*Right*) or absence (*Left*) of IL-7 (from $n = 3$ experiments with ≥3 thymic lobes per condition).

they can easily be found in the thymus of adult mice, where they express conspicuously high levels of IL-7R (Fig. 4 *A* and *B*). High levels of *Il17* mRNA were consistently detected only among IL-7R^{hi}γδ^{27-} cells (Fig. 4*C*). IL-7 is absolutely required for γδ cell development, and culture with IL-7 fueled the survival and expansion of all adult γδ thymocytes, as shown by a time course (Fig. S3*A*). Nonetheless, there was again a strong enrichment for CD44^{hi}IL-17–competent, TCR^{hi} CD27^{-} γδ cells, with such cells transitioning from the minority to the majority by comparison to IFN-γ–competent cells (Fig. 4*D* and Fig. S3*A*).

As for LN cells, CFSE labeling and Ki67 staining of thymocytes ex vivo showed that IL-7 primarily promoted proliferation of CD44^{hi}CD27^{-} thymocytes with IL-17 potential (Fig. S3 *B* and *C*): Indeed, after 4 d, >90% of CD44^{hi}CD27^{-} thymocytes were cycling with >98% of IL-17–competent cells found among these. Again there was little evidence for IL-7–mediated selective survival of γδ^{27-} cells with *Bcl-2* mRNA levels lower in γδ^{27-} cells than in γδ^{27+} cells (Fig. S3*D*). The preferential expansion of CD44^{+}γδ^{27-} thymocytes was reduced ≥80% by the STAT3 inhibitor (Fig. 4*E* and Fig. S3*E*). Conspicuously, thymic γδ^{27-} cells were not selectively enriched by other STAT5- (IL-2, IL-15, and IL-21) and STAT3- (IL-6) activating cytokines, either alone or added to suboptimal concentrations of IL-7. IL-2 activated all γδ thymocytes, but still enriched (almost twofold) for γδ^{27+} cells, whereas IL-15 primarily activated γδ^{27+} cells (Fig. S4). Thus, as for LN cells, IL-7 promotes preferential STAT3-dependent expansion of IL-17–competent γδ^{27-} thymocytes.

Further evidence that IL-7 preferentially expands IL-17–competent thymocytes within the γδ^{27-} subset was derived from the Il17aCreR26ReYFP mice. Ex vivo ∼1% of γδ thymocytes are eYFP^{+}CD27^{-}, whereas ∼8% are eYFP^{-}CD27^{-} (Fig. 4*F*), roughly consistent with ∼11% of γδ^{27-} thymocytes producing IL-17 upon short-term activation (Fig. 4*D, Upper*). Conversely, 4 d in IL-7 increased eYFP^{+}CD27^{-} cell numbers >30-fold, making them the larger subset compared with eYFP^{-}CD27^{-} cells that had increased much less (Fig. 4*F*).

To demonstrate that developing γδ^{27-} cells are a preferential target of IL-7, fetal thymocytes were examined because the fetal thymus will not be a target for peripheral T-cell recirculation. Supplemental IL-7 added to 7-d fetal thymic organ culture (FTOC) expanded absolute numbers of total γδ thymocytes by ∼fivefold, but again the impact was preferential for IL-17–competent γδ thymocytes whose representation was increased twofold over IFN-γ–producing γδ thymocytes (Fig. 4*G*). To verify that IL-7 preferentially activated γδ^{27-} thymocytes rather than promoting the conversion of γδ^{27+} thymocytes to γδ^{27-} cells, IL-7 was applied to purified CD44^{hi}γδ^{27-}, CD44^{hi}γδ^{27+}, or CD44^{lo}γδ^{27+} thymocytes. After a 4-d culture, CD44^{hi}γδ^{27-} cells appeared by microscopy to be highly activated, by contrast to the γδ^{27+} subsets. To normalize the number of cells in the cultures, purified subsets were admixed with thymocytes from age-matched TCRδ$^{-/-}$ mice. Strikingly, neither γδ^{27+} subset generated IL-17–competent cells over 4 d, whereas >70% of cells arising from only 5,000 CD44^{hi} γδ^{27-} were IL-17 competent (Fig. S5). Thus, IL-7 primarily expands cells with IL-17 competence rather than differentiating cells toward IL-17 de novo.

IL-7 Promotes Adaptive Potential to Produce IL-17. IL-17–producing γδ cells are widely viewed as innate because they are rapidly activated by IL-1 and IL-23 alone and are relatively unresponsive to TCR agonists that strongly activate IFN-γ–producing γδ^{27+} cells (Fig. S6*A*) (9). However, in the presence of IL-7, TCR agonists promoted a >20-fold enrichment of γδ^{27-} cells relative to LN cells, whereas γδ^{27+} cells were enriched by only three- to fourfold: By 4 d, ∼100% of γδ^{27-} cells were CD69^{+}CD44^{hi}CD25^{+} ICOS^{+} (Fig. 5 *A* and *B* and Fig. S6*B*). Compared with IL-7 alone, suboptimal concentrations of TCR agonists added to IL-7 increased γδ^{27-} IL-17–competent cell numbers by an additional 40–50% (Fig. 5 *C* and *D*), whereas there was negligible synergy for γδ^{27+} cells, which instead responded very strongly to the combination of IL-15 + TCR agonists (Fig. S6 *C* and *D*).

As a preface to killing target cells in response to TCR-mediated activation, T cells exocytose the contents of cytolytic granules in a process that involves movement to the cell surface of the protein CD107a (27). Hence, CD107a expression levels provided an additional assay for the impact of IL-7 on the response of γδ T cells to TCR stimulation. Strikingly, TCR agonists provoked surface up-regulation of CD107a by γδ^{27-} cells only after the cells' culture in IL-7, whereas γδ^{27+} cells up-regulated CD107a expression directly ex vivo, with no requirement for IL-7 (Fig. 5*E*). In sum, IL-7 selectively facilitates strong responses of IL-17–producing γδ cells to TCR stimulation whether measured by expansion, activation markers, or effector function.

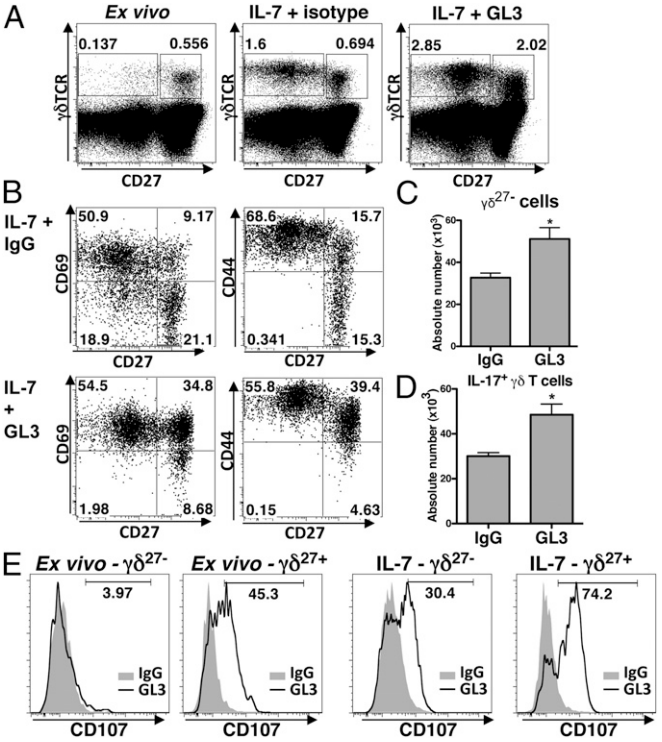

Fig. 5. TCR agonists and IL-7 cooperatively promote IL-17–producing γδ cells. (*A*) Total LN cells stained for markers as indicated ex vivo (*Left*) and after 4-d culture with IL-7 + 1 μg/mL TCR-agonist antibody [GL3] (*Right*) or isotype control (*Center*). (*B*) LN γδ T cells stained for markers as indicated after 4-d culture with IL-7 and either TCR-agonist antibody (*Lower*) or isotype control (*Upper*). For all plots, numbers indicate percent of cells in relevant quadrants. (*C*) Absolute number of total γδ^{27-} (*Upper*) or IL-17–expressing γδ^{27-} (*Lower*) LN cells after culture in IL-7 with 1 μg/mL GL3 or IgG control, as in *A*. Error bars are SEM from $n = 3$ experiments; $^{*}P < 0.05$. (*E*) Staining for CD107 in gated γδ^{27-} (*Left*) and γδ^{27+} (*Right*) cells, activated for 6 h with 10 μg/mL TCR agonists (line) or isotype control (shaded area), from ex vivo LN (*Left*) and after 4-d culture with IL-7 (*Right*).

IL-7 Reveals IL-17–Competent Human γδ Cells. By contrast to mice, a substantive subset of IL-17–producing human γδ T cells has been hard to identify in healthy donors (16, 28). As reported (29), there was precocious production of IFN-γ by fresh human cord blood (CB) γδ cells and by adult TCRγδ^{+} peripheral blood mononuclear cells (PBMC) stimulated by PMA + ionomycin, but there was no obvious IL-17–producing subset (Fig. 6*A*). When PBMC were cultured for 1 wk with anti-TCRγδ + IL-7 and then activated for 6 h, IFN-γ monoproducers described ∼80% of cells; a small percentage coexpressed IL-17 and IFN-γ, but there was still no IL-17 monoproducer (Fig. 6*B*). However, when CB cells were likewise cultured, substantial fractions of Vδ2^{+} and Vδ1^{+} cells produced IL-17 with most being IL-17-monoproducers (Fig. 6*B* and Fig. S7*A*). Unsurprisingly, the percentages of γδ cells that were IL-17-competent varied with the source of CB from ∼15% to >40%, with higher representation always being among Vδ2^{+} cells: Indeed, IL-17–competent Vδ2^{+} cells sometimes outnumbered

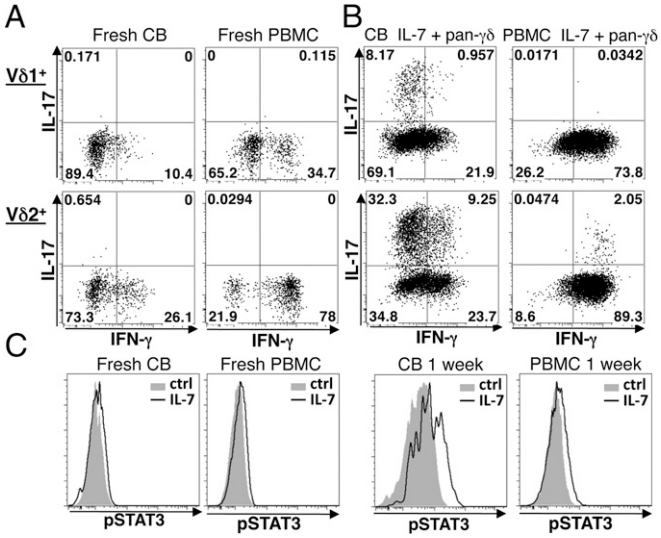

Fig. 6. Evocation of IL-17–producing γδ T cells from human cord blood. Representative plots from $n \geq 4$ donors. (*A* and *B*) Intracellular staining for IFN-γ and IL-17 in Vδ1$^+$ (*Upper*) and Vδ2$^+$ (*Lower*) cells from CB and adult PBMC freshly isolated (*A*), or cultured for 1 wk (*B*) with IL-7 and pan γδTCR antibody: In each case, cells were prestimulated with PMA + ionomycin for 6 h. (*C*) pSTAT3 and pSTAT5 levels assessed by flow cytometry in gated Vδ2$^+$ cells from fresh CB or adult PBMC (*Left*), or after 1 wk of culture with anti-CD3 + IL-2 (*Right*), in each case followed by 30-min IL-7 stimulation (open areas) or control (ctrl, shaded areas).

IFN-γ–competent cells (Fig. 6*B* and Fig. S7*B*). Comparing the main γδ cell subsets: 1-wk culture with IL7 + TCR agonists sustained absolute numbers of Vδ1$^+$ cells but IL-17 monoproducers expanded by ∼50-fold, compared with no significant increase in IFN-γ–competent cells, whereas Vδ2$^+$ cell numbers increased ∼10-fold within which IL-17 monoproducers expanded by >20-fold, compared with only a 10-fold increase in IFN-γ–competent cells. Hence, as in the mouse, the effects of IL-7 + TCR agonists are strongly biased toward IL-17 competence. Consistent with γδ cell activation, ∼100% lost the naïve T-cell marker CD45RA, up-regulated ICOS, and expressed CD161, a marker of human IL-17–producing T cells (30) (Fig. S7 *B* and *C*). When fresh or 1-wk cultures of CB or PBMC were freshly stimulated for 30 min with IL-7, all showed increased STAT5 phosphorylation, which did not therefore correlate with IL-17 competence. Conversely, STAT3 activation was measurable only in CB-derived Vδ2$^+$ cells when cultured for 1 wk and then stimulated with IL-7 (Fig. 6*C* and Fig. S8*A*). Thus, as in mouse, IL-7–dependent STAT3 activation is highly selective.

CB Vδ2$^+$ cells cultured for 1 wk with IL-7 + TCR agonist and stimulated for 2 h with PMA + ionomycin showed conspicuous reductions in RNA for T-bet, IL-2, and IFN-γ, with corresponding increases in IL-17A, IL-17F, and IL-22 RNAs (Fig. S8*B*). Indeed, a small number of IL-22–expressing cells was evoked, with the majority coproducing IL-17 (Fig. S8*C*). Again, these results were specifically due to IL-7, because IL-2 + TCR agonists skewed CB cells toward IFN-γ, with ∼10-fold fewer cells expressing IL-17 (Fig. S8*D*).

Discussion

This study identifies a pathway selectively activating the production of IL-17 by γδ T cells. It operates in vivo and is conserved in mice and humans. It broadens our perspectives on the biology of IL-7 and has clinical relevance, given that IL-7 is used to promote lymphocyte expansion in cancer patients and in bone marrow transplantation (31) and given that protocols are being sought to maintain and expand γδ cells for adoptive immunotherapy. Conversely, IL-7 blockade is being considered for inflammatory diseases.

Although a specific cytokine may not be essential for the development and survival of a particular lymphocyte subset, its capacity to regulate such cells is important. Such is the case for IL-7 and memory CD8$^+$ αβ T cells, which are unaffected by IL-7 depletion but are nonetheless substantially expanded by IL-7, evoking an accumulation of CD44^{hi}CD8$^+$αβ T cells (32, 33). Likewise, IL-17–producing γδ cells may not depend on STAT3 (15), but they are rapidly responsive to IL-23 that signals via STAT3 (10). In this vein, this study identifies a capacity of IL-7 to activate STAT3 preferentially in γδ cells competent to produce IL-17, markedly expanding and activating such cells and promoting their functional responsiveness, for example, increased cytolytic potential. This activation is a selective role for IL-7 that for γδ cells has hitherto been regarded as a generic regulator of development and homeostasis for all γδ subsets (17, 34). This role of IL-7 offers parallels with its reported requirement to maintain TCRαβ$^+$ Th17 cells, although a key difference is that the effects of IL-7 in that case were mediated by STAT5 (35), which seems paradoxical given that STAT5 can antagonize Th17 differentiation. By contrast, the role of IL-7 described here is in large part mediated by STAT3, emphasizing the importance of a relatively poorly understood phenomenon—namely, how different cytokines use different signaling pathways to effect specific roles.

Like the effects of IL-7 on αβ T cells (36, 37), IL-7–induced γδ^{27-} expansion in vivo occurs in the periphery, but unlike for αβ T cells, this expansion does not require prior lymphodepletion, emphasizing the capacity of IL-7 to act selectively on γδ^{27-} cells in physiologic situations. Indeed, blocking IL-7R almost completely abrogated the expansion of LN IL-17–producing γδ cells in response to IMQ, although it left the expansion of IFN-γ–producing cells largely untouched. Correspondingly blocking IL-7R inhibited the development of inflammatory lesions. Furthermore, increased IL-7 levels are detected in vivo after TLR activation (38) and in autoimmune diseases, such as rheumatoid arthritis (39), systemic juvenile rheumatoid arthritis (40), multiple sclerosis (41), and psoriasis (42), suggesting scenarios where potential dysregulation of IL-17-competent γδ cells should be investigated. Indeed, IL-17–producing γδ cells were recently described in psoriatic skin (11, 20, 43).

γδ cells are not confined to T-cell zones and may access reticular stromal cells that express IL-7 (44), potentially in the context of tissue-draining antigens. Thus, the capacity of IL-7 to promote γδ^{27-} cell activation in response to TCR agonists provides an important perspective on cells commonly assigned to innate immunity. Additionally, we have shown that IL-7 selectively expands CD44^{hi} γδ^{27-} cells from the peritoneum, where CD44^{hi} γδ T cells are strongly implicated in defense against bacterial infection (45).

Finally, IL-7 appears most abundant in neonatal mice, which is when lymphoid compartments are being filled according to homeostatic mechanisms and when murine IL-17–producing γδ cells, largely derived from fetal progenitors, are most abundant. Indeed, IL-7 may throughout life mobilize IL-17–producing γδ cells from a "self-renewing" pool set down in the fetus. It is also striking that the major evocation of human IL-17–producing γδ cells is from CB. Interestingly, two studies using cytokines established to promote Th17 cells used CB to evoke small numbers of IL-17–producing γδ cells (46, 47). γδ cells are functionally precocious relative to αβ T cells in mice and in humans, and one of their major biological contributions may be to protect neonates (29, 48, 49). Moreover, inflammatory immunopathologies may reflect inappropriate mobilization of cells laid down in the fetal/neonatal period. In pursuing this hypothesis, the major and selective potential of IL-7 needs now to be considered in immunoprotection and immunopathologies. This study also implies reciprocal selective roles of IL-2 and IL-15 in regulating IFN-γ–competent γδ cells, which may be germane to the resurgent clinical use of these reagents.

Materials and Methods

Cell preparation, flow cytometry, PCR, and cytokine measurements were performed as described in *SI Materials and Methods*.

Murine and Human Samples. For methods related to animals and human samples, see *SI Materials and Methods*. In some experiments, mouse in vivo i.p. injections included PBS or recombinant mouse IL-7 [rmIL-7 (R&D Systems), 5 μg per mouse every 2 d for 1 wk]. In other experiments, a daily dose of 50 mg of Imiquimod (5% IMQ cream; Meda AB) or control cream (Vaseline) was applied to shaved backs of mice for 3 d. Anti–IL-7R (clone A7R34) or rat IgG control treatment was performed by i.p. injection (1 mg per mouse) on days −1 and +2 relative to IMQ application. A7R34 was obtained from Biolegend or, for some experiments, we made A7R34 from hybridoma (50).

Cell Culture. Cells were incubated for 1, 2, 3, or 4 d with IL-2 (100 U/mL; Immunotools), IL-7, IL-6, IL-15, and IL-21 (all 20 ng/mL; R&D Systems). Where indicated, anti-TCRγδ (GL3: 1 or 10 μg/mL), IgG1κ isotype control, and anti-CD107a/b antibodies (1D4B, M3/84; Biolegend) were also added. After culture, dead cells were removed by Ficoll-Hypaque centrifugation (GE Healthcare). In some experiments, cells were preincubated with STAT3 inhibitor VII (Calbiochem) for 1 h before addition of IL-7. For human studies, cells were cultured for 1 wk with IL-7 (20 ng/mL; R&D Systems) or IL-2 (100 U/mL; Immunotools) in wells coated with pan anti-γδTCR (1 μg/mL, IMMU510; Beckman).

ImageStream Acquisition and Analysis. Samples (4×10^7 cells per mL in 60 μL of wash buffer with 1 μg/mL PI) were acquired on a 5-laser 6-Channel ISx Imaging Flow Cytometer with 40× magnification controlled by INSPIRE software and fully ASSIST calibrated (Amnis).

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